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CORRELATION BETWEEN THE HYDRATION OF ACYL CHAINS AND PHOSPHATE GROUPS IN LIPID BILAYERS: EFFECT OF PHASE STATE, HEAD GROUP, CHAIN LENGTH, DOUBLE BONDS AND CARBONYL GROUPS.

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ABSTRACT.

This paper demonstrates by means of FTIR/ATR analysis that water molecules intercalate at different extents in the acyl chain region of lipid membranes in correlation with the hydration of the phosphate groups.

This correlation is sensible to the chain length, the presence of double bonds and the phase state of the lipid membrane.

The presence of carbonyl groups CO modifies the profile of hydration of the two regions as observed from the comparison of DMPC and 14:0 Diether PC.

The different water populations in lipid interphases would give arrangements with different free energy states that could drive the interaction of biological effectors with membranes.

Keywords: lipid membranes; hydration; lipid structure, phase state; FTIR/ATR spectroscopy.

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Abbreviations: 1,2-dimyristoyl-*sn*-glycero-3 phosphocholine (DMPC); 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC); 1,2-di-O-tetradecyl-*sn*-glycero-3-phosphocholine (14:0 Diether PC); 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE); 1,2-dipalmitoleoyl-sn-glycero-3-phosphocholine (16:1 (Δ9-Cis) PC); relative humidity (RH).

INTRODUCTION.

Phosphatidylcholine (PC) is the prevalent lipid component in eukaryotic cell membranes. Four structural regions can be distinguished: the phosphatidylcholine moiety, the glycerol backbone and two acyl chains esterified in position sn_1 and sn_2 of different length and saturation. In turn, the phosphatidycholine region is composed by a negatively charged phosphate group bound to a positive choline group, which is rather hydrophobic [1-3]. In an excess of water, bilayers are stabilized with a defined area per lipid and thickness [4-6]. The surface area per lipid and the hydrocarbon chain order parameter govern various dynamical membrane properties such as lateral diffusion, compressibility and permeation. Most of these properties are strongly dependent on the hydration state of the lipids [7, 8].

Water is, thus, a key element in a variety of structural and functional roles in membranes of living cells that takes relevance when cells suffer hydric, thermic or salt stress [1]. Coupling phenomena between membranes, such as adhesion, fusion, and stacking, depend on hydration forces derived from the restructuring of water [9][10]. How and which hydration levels are involved in each of these processes is still a matter of debate. The discussion on the stability of the different water arrangements in the hydration shell and hence their thermodynamic properties that could make feasible the interactions is open.

For this reason, in the search of a general pattern of behaviour, the understanding of the structural role of water in lipid membranes have received considerable attention since Luzzatti et al.[2] first determined by SAXS the thickness of lipid bilayers. After this discovery, efforts have been addressed to give a defined location to water and to determine the appropriated values for area per lipid molecule [7, 8, 11, 12].

The consideration of water as a structural component of biological membranes, specifically its thermodynamic properties at the interphase, is relevant not only to define bilayer structural parameters in fully hydrated states, but mostly because in several physiological processes*, hydration-dehydration* steps play a crucial role. This

accounts for the interdependence of the hydration degree and dynamics of different moieties of the different phospholipids during osmotic and hydric stress, cell aging and interaction of biological effectors with the membrane [13]

In the first stages of the hydration process, several water molecules interact with the PO_2^- groups mainly in a first layer array [14]. These groups are sensible when hydration levels fall around six water molecules for PC's and hence they are relevant in physiological processes when a drastic dehydration is imposed [15, 16].

After saturation of PO_2^- [17, 18] and CO headgroups [19] in PC´s, water molecules assemble in the form of a clathrate hydration shell around the hydrophobic $N(CH_3)^{3+}$group [20-24]. It has also been reported that water may penetrate into the first carbon atoms below the carbonyl plain in the hydrocarbon region forming different clusters of water populations [5, 6, 25] changing the orientation of carbonyl groups [26, 27] and the conformation of the acyl chains [28].

Further studies with FTIR spectroscopy showed that CO groups at the sn_1 and sn_2 acyl chains show unbound and bound populations. The first one at higher frequencies identifies the CO groups oriented toward the hydrocarbon core and the other, at lower frequencies, those facing the aqueous phase with which it forms H bonds. The presence of both populations at each chain can be explained by a dynamic picture in which CO of the sn_1 and sn_2 chains alternate between hidden and exposed positions to water depending on membrane topology [29] [30]. Other works using FTIR spectroscopy have shown that the CH_2 frequency groups in the acyl chains of different phospholipids increases significantly at the phase transition temperature [31]. The comparative analysis of the frequencies for different phospholipids in terms of reduced temperature gives as a result that the CH_2 frequency groups were similar in the gel state for the different lipids. In contrast, there was a pronounced difference above the transition temperature correlated with the length, saturation and branching of the acyl chain [32]. The highest values were obtained for lipids with unsaturation and branching and were related to the rotational isomers of the hydrocarbon chains forming kinks as postulated by Traüble [33].

The interpretation of the changes in frequencies allows to postulate two kinds of CH_2 populations in the acyl chains. One of them, corresponding to low frequency values, is found in the gel phase and denote CH_2 residues that are in contact with its immediate neighbours by dispersion forces. This CH_2-CH_2 contact produces a decrease in the CH bond frequency due to the weakening of the bond strength [34].

Above the transition temperature, chains have, in average, a lower contact due to thermal agitation and the water per lipid ratio increases. Thereby the isolated populations are visualized by the high frequency values since the CH bond is stronger in the absence of lateral contacts. In the liquid crystalline state the isolated CH_2 groups were separated by water molecules between them [33-35]. However, no direct evidence of the amount of water in the bilayer and the modification of the ratio between connected and isolated populations in the acyl chain region with the hydration of other groups has been up to now reported.

The appearance of water in between the chains results in a different vision of the lipid bilayer classically thought as a hydrocarbon slab in which polar solutes are unable to dissolve. Moreover, water content can be modulated by the chemical features of the lipid constituents [36, 37]. This approach appears to be congruent with the states of the membrane in which it becomes “reactive” for solutes in the aqueous media [33, 38-40]

Therefore, this paper analyses by FTIR the hydration of acyl chains in correlation with the hydration of the phosphate groups and its influence on the molecular structure of the phospholipids and phase state. In particular, the acyl chain length, the polar head groups, the introduction of double bonds and the presence of carbonyl groups were specifically taken into account.

In order to mimic the hydric stress, the degree of hydration of the bilayers was changed by exposing the lipids to different relative humidities. The results allow comparing the water level and its distribution in different parts of the phospholipid molecules.

EXPERIMENTAL SECTION.

Lipids and chemicals

1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC- 16:0); 1,2-dimiristoyl-*sn*-glycero-3-phosphocholine (DMPC- 14:0); 1,2-di-O-tetradecyl-*sn*-glycero-3-phosphocholine (14:0 Diether PC); 1,2-dipalmitoleoyl-sn-glycero-3-phosphocholine (16:1 (Δ9-Cis) PC); 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE 14:0) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL). Purity of lipids were higher than >99% as checked by FTIR (see Figure S1) and UV spectroscopies.

ATR-FTIR Spectroscopy

All ATR-FTIR spectra were obtained in a Thermo Scientific 6700 spectrometer assembled with an ATR accessory with humidity and temperature chamber control, and DTGS KBr detector, connected to a system of circulation of dry air to avoid the interference of water vapor and carbon dioxide. Films were prepared from chloroformic lipid solutions by evaporating the chloroform solvent under nitrogen stream and 24 h vacuum. Then, they were resuspended, above the transition temperature, in a minimum volume of MQ water to reach a 20 mM lipid suspension. Droplets (2 µL) of each suspension were placed on diamond crystal (45° incident angle) and exposed to air of selective relative humidities. Initial spectra of fully hydrated samples were taken at temperatures below and above the phase transition for each of the lipids assayed. Then, samples were allowed to stabilize at chosen relative humidities at all temperatures [41, 42]. The relative humidity was controlled throughout the measurements by using saturated salt solutions of LiCl, NaCl and distillated water to reached 11, 75 and 100 %RH respectively.

Data were obtained after 64 scans per sample at a 4 cm^{-1} resolution corresponding to the average of three independent assays. Spectra were obtained in intervals of three minutes in order to control the water content evolution following the water band intensity and the asymmetric stretching vibration of the methylene groups ($\nu_{as}CH_2$). Spectra were analyzed with Omnic Software (version 9.1.24) and Microcal Origin program (version 8.5). These softwares mathematically process the spectra and the peak maxima were determined by the Omnic find peak function routine resulting in an accuracy of 0.1 cm^{-1} which gives statistically reliable data [43, 44]. The complete FTIR/ATR spectra of pure DPPC at different RH are shown in Figure S2 (Supporting Information). From these results, the evolution of the water, asymmetric CH_2, and symmetric PO_2^- bands were inspected at different vapour pressures.

Membrane phase behavior was determined by the evolution of the asymmetric CH_2 stretching vibration band (approximately at 2918 cm^{-1}) arising from the lipid acyl chains, and the symmetric PO_2^- stretching band (approximately at 1085 cm^{-1}) arising from the lipid head groups. The symmetric PO stretching band in our hands gives a clear information because it presents a narrow and well defined peak for all the lipids and conditions assayed in contrast to the asymmetric PO_2^- vibration band that shows a complex composite band for DMPE, DMPC (18 °C) and DPPC (22 °C).

Asymmetric CH_2 in spite of being more sensitive to temperature, it shows more noticeable frequency variations along hydration. For this reason, care was taken to carry out all experiments at controlled temperature within ± 0.1 ºC.

Relative humidity of 11% was chosen as the optimal atmosphere in order to follow the evolution of the system from fully hydrated state to the lowest value of water activity values that can be achieved in these experimental conditions.

The same process, carried out with different relative humidities such as 100%, 75% and others, shows an evolution picture that overlaps with that obtain at 11% but those processes give less information because the equilibration is reached at higher water levels as shown in Figure 1B.

State diagrams were obtained by plotting the wavenumber position of the lipid $\nu_{as}CH_2$ or $\nu_sPO_2^-$ stretching band as a function of water content defined as $I\nu H_2O/I\nu CH_2$. This ratio was chosen because in this manner we can analyze the hydration states of all studied lipids including those lacking CO groups. Previous works showed that measurements for the water content also take into account other vibrational modes such as water scissoring band and the lipid ester band [44].

RESULTS

The relation of the intensity of the water band and the intensity of methylene groups ($I\nu H_2O/I\nu CH_2$) is a direct measure of the hydration level in the lipid dispersion as observed in Figure 1A. This relationship decreases to different equilibrium values when DPPC at 22°C ± 0.1 is exposed to 100%, 75% and 11% RH atmospheres. As expected, the time to reach equilibrium decreases when exposed to lower relative humidity, i.e. the higher is the difference in chemical potential of water between the sample and the atmosphere.

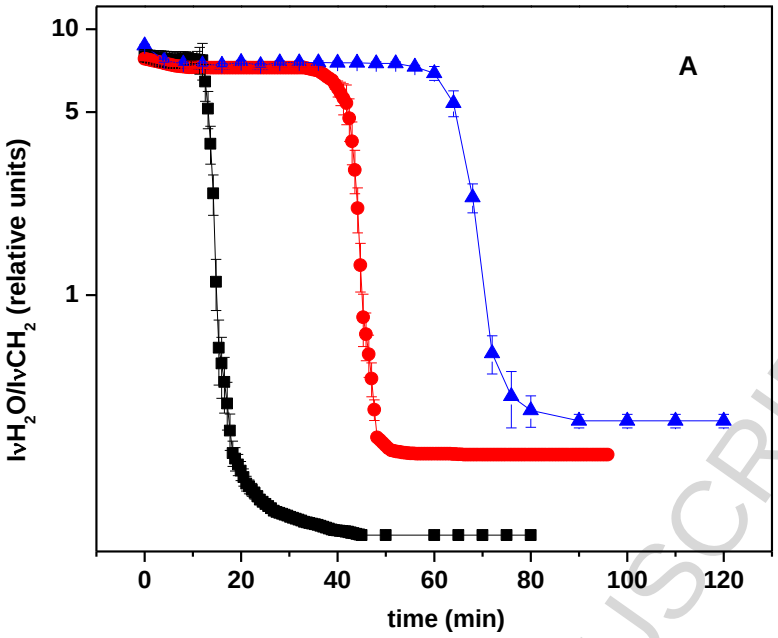

Figure 1 (A): Evolution of the water content in DPPC bilayers during equilibration in 100% (blue triangle); 75% (red circle) and 11% (black square) relative humidities (RH) at 22 °C ± 0.1.

The shape of the water band along the evolution of water content in the lipid matrix changes according to lipid phase and stages of hydration level as reported in previous papers [45, 46]. The water bands corresponding to Figure 1A are shown in Figure S3 in Supporting Information.

In Figure 1B, the simultaneous change in the frequency of the $\nu_{as}CH_2$ residues and the phosphate groups along the water content is shown. The equilibration progress to 100% (red dots) and 11% (black dots) overlaps in the first stage in which $\nu_{as}CH_2$ frequency decreases from 2918.2 until 2916.8 cm^{-1} without significant variations in the phosphate ones (1089.0 cm^{-1}). In a further stage of dehydration below $I\nu H_2O/I\nu CH_2$ = 0.33 the change in water content affects both $\nu_{as}CH_2$ and $\nu_sPO_2^-$ groups.

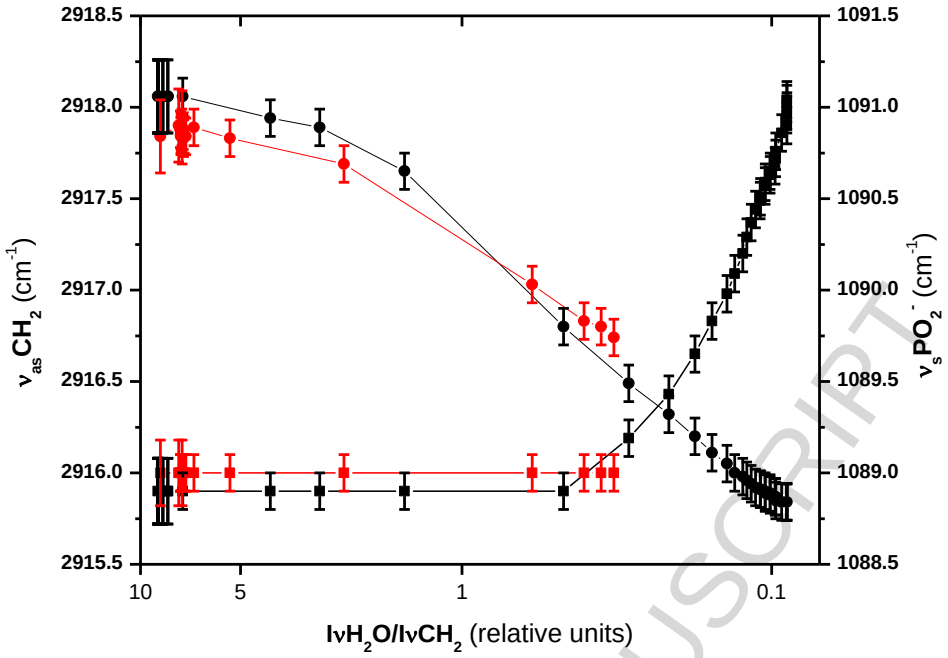

Figure 1 (B): Effect of water content on the $\nu_s PO_2^-$ (black circles for 11% RH and red circles for 100% RH) and $\nu_{as} CH_2$ (black squares for 11% RH and red squares for 100% RH) at 22 ºC ± 0.1 for DPPC.

In order to compare the effect of the phase state in the same lipid, the equilibration process at 11% RH was performed above (50 °C) and below (22 °C) the phase transition temperature of DPPC (T_c = 41°C) (Figures 2A and B).

In Figure 2A, the dehydration process of DPPC at 22 °C ± 0.1 (blue dots) promotes a continuous decrease in the frequency of $\nu_{as} CH_2$ and an increase of that corresponding to phosphate. However, at 50 °C, the same process show a shift to lower $\nu_s PO_2^-$ frequencies and a discontinuity at around $I\nu H_2O/I\nu CH_2$= 1.

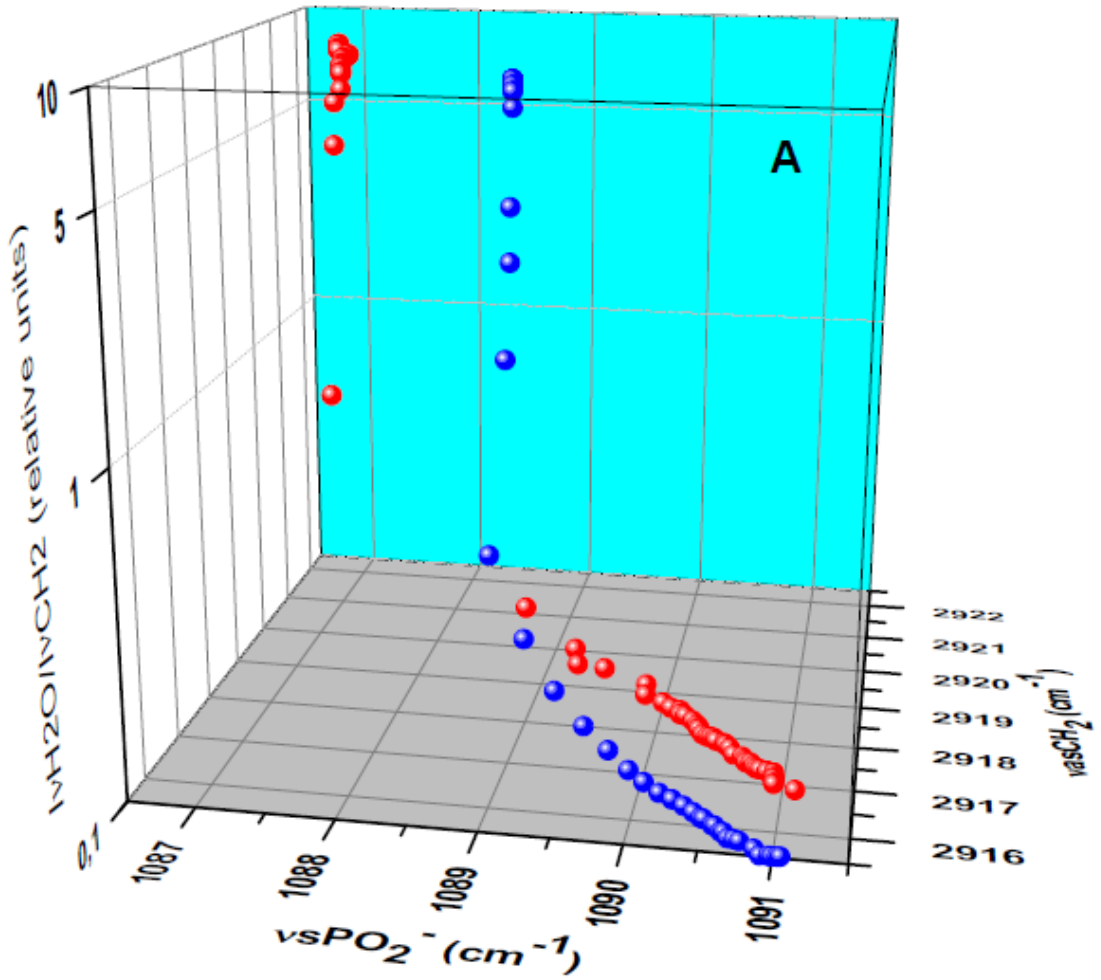

Figure 2 (A): 3D diagram of the $\nu_s PO_2^-$ and $\nu_{as}CH_2$ equilibration at a hydration level of 11 %, below (22 °C – blue circles) and above (50 ºC – red circles) the phase transition temperature of DPPC.

Figure 2B shows in more detail this process. At the low hydration region, the changes in the acyl chains and in the phosphate groups run parallel below and above the phase transition temperature although the frequency of $\nu_{as}CH_2$ is displaced to higher values at 50 °C (red points). At 22 °C, phosphate frequencies reached a constant value at 1088.9 cm^{-1} in spite that changes in the acyl chains are significant. At 50 °C the saturation of the phosphate groups appears to be displaced to much lower values (c.a. 1087.0 cm^{-1}) suggesting a change in the phosphate groups hydration state. Between 1090 cm^{-1} and 1089 cm^{-1}, $\nu_s PO_2^-$ frequencies decrease without changes in the CH_2 region. This region is coincident with the lyotropic transition observed at that temperature in the temperature/water content phase diagram as reported before [38]. Taking into account these considerations,

the inspection of the data for DPPC at 50 °C in Figure 2B indicates that at high water contents, the phosphate frequency does not change with the decrease of CH_2.

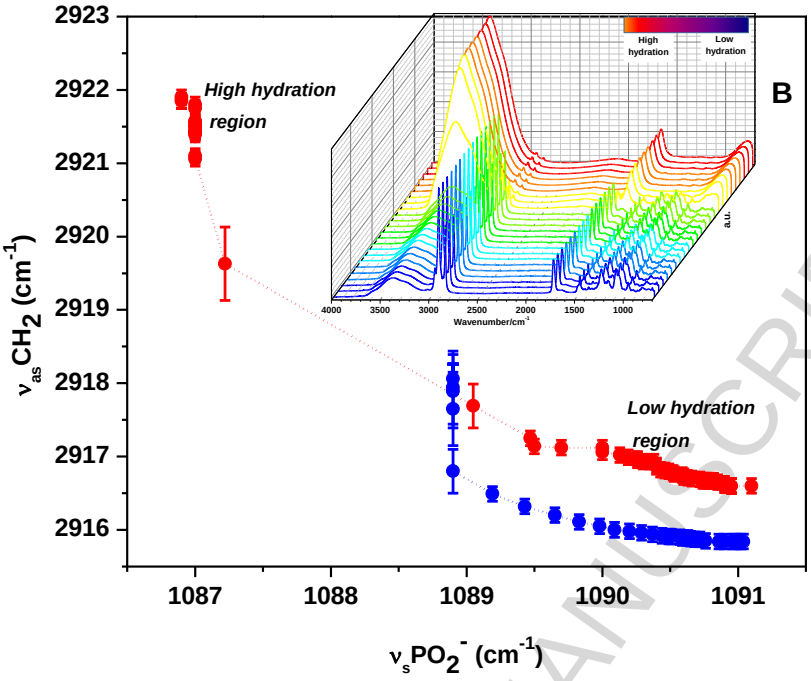

Figure 2(B): Relation between two levels of hydration (low-high) assessing the frequencies below (22 °C – blue circles) and above (50 ºC – red circles) the phase transition temperature of DPPC.

A better inspection of the simultaneous changes occurring in the acyl chains and the phosphate groups with the hydration level is given in Figure 3 for DPPC at 22 °C (part A) and at 50 °C (part B). In first place, it must be noticed that the process described in Figure 3A occurs in phase diagram region in which no changes of the phase state takes place i.e. lipids remain always in the L_{β}' phase. Dehydration process of the phosphate groups proceeds from 1089.2 cm^{-1} until 1091 cm^{-1} which corresponds to the lower water content achieved al 11 %.

A significant change in the acyl chains region occurs at c.a. $I\nu H_2O/I\nu CH_2 = 3$. At this point CH_2 frequency starts decreasing although phosphates frequencies remain constant. When $I\nu H_2O/I\nu CH_2$ is lower than 0.5 an increase in phosphate frequency is observed indicating strong dehydration of the phosphates meanwhile CH_2 continues decreasing gradually, suggesting that these two hydration sites are independent. This point corresponds to the observation in Figure 2B showing that phosphates remains constant at 1089 cm^{-1} with a significant variation in CH_2 frequency.

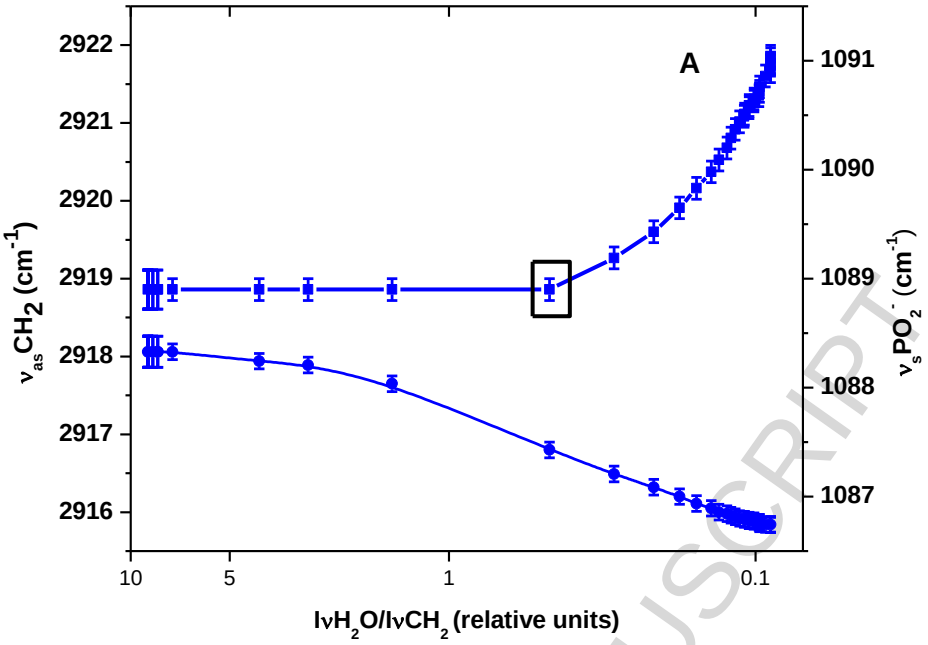

Figure 3 (A): Effect of water content on the $\nu_s PO_2^-$ (blue squares) and $\nu_{as}CH_2$ (blue circle) of DPPC in gel phase (22 °C).

The same analysis made for DPPC at 50 °C (Figure 3B) indicates that phosphate frequency increases from 1087.0 to 1091 cm^{-1}. The lower frequency of phosphates at high level of water content in comparison to that found at 22 °C suggests a higher hydration of phosphates at 50 °C. On the other hand, there was a higher CH_2 initial frequencies related to a major number of isolated acyl chains populations that generates free spaces where water molecules can be inserted [34, 35]. When the CH_2 frequency is lower than 2921 cm^{-1} the phosphate groups and the acyl chain frequencies noticeable vary simultaneously during the process. From 1089.5 cm^{-1} to 1090 cm^{-1} for phosphate groups, the $\nu_{as}CH_2$ is constant. From this point, acyl and PO_2^- frequencies vary again in a concomitant way. The plateau at the CH_2 frequency reflects that a transition takes place in which no change in the acyl chain region occurs during phosphates dehydration. The beginning of the plateau (1089.5 cm^{-1}) suggests that the phosphates affinity for water predominates in comparison to that for the acyl region. This change could be due to the lytropic transition from the L_α to the L_β phase observed in the temperature water content phase diagram. This is congruent with that reported by Binder et al [47].

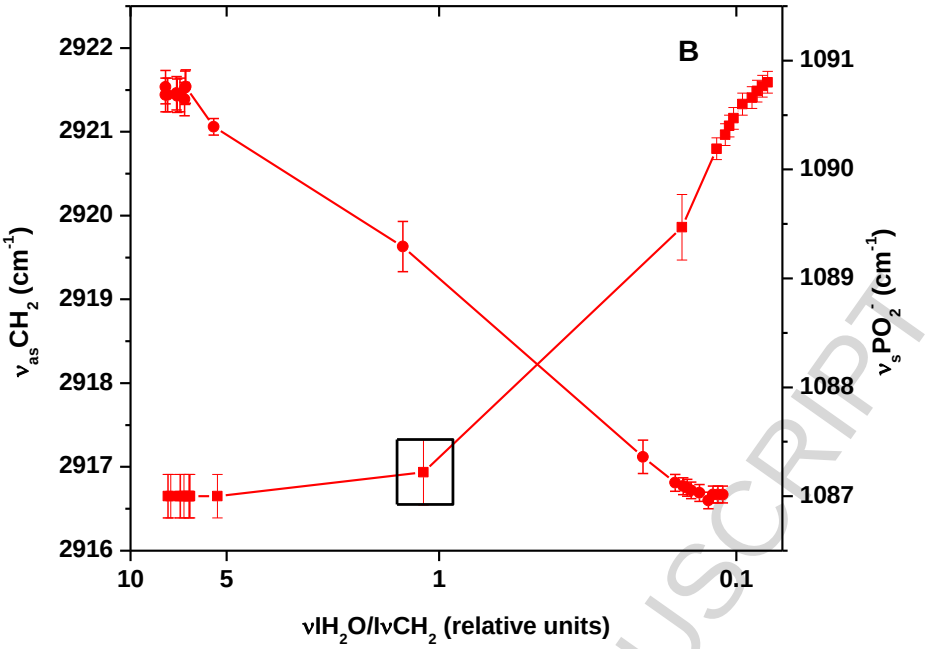

Figure 3(B): Effect of water content on the $\nu_s PO_2^-$ (red squares) and $\nu_{as} CH_2$ (red circle) of DPPC in the liquid crystalline phase (50 °C).

The profile of phosphate groups and acyl chains hydration levels in the same phase state and same chain length for saturated and unsaturated chains are compared in Figure 4. It is observed that the presence of a double bond at position 9 (16:1 (Δ9-Cis) PC) introduces drastic changes in the profile. The frequency of CH_2 groups remains at around 2923.0 cm^{-1} in the whole range in which phosphate frequency changes from 1085.5 to 1090 cm^{-1}. This suggests a low interaction between the acyl chains even at the lowest level of hydration of the phosphate groups. The double bond would hinder the packing along the whole length of the acyl chains. It must be noticed that according to the phosphate frequency values the hydration of the phosphates follows the sequences DPPC 20°C < DPPC 50°C < DOPC 22°C, denoting that acyl chain nature affects the water access to phosphates.

To put on relevance the interdependence of the hydration at PO_2^- and acyl chain levels, it was of interest to analyse it in lipids lacking carbonyl groups, since these are also hydration sites.

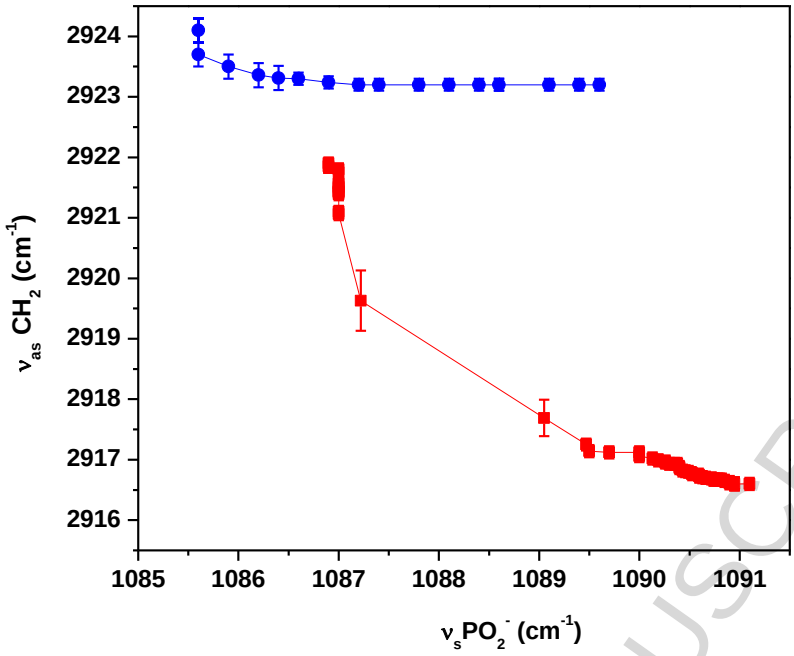

Figure 4: Correlation of $\nu_s PO_2^-$ and $\nu_{as} CH_2$ equilibrated at 11% RH for DPPC at 50 °C (red squares) and 16:1 (Δ9-Cis) PC at 22 °C (blue squares).

In Figure 5A, it is observed that the CH_2 frequency decrease (blue pentagons) of the 14:0 Diether PC (Tc=26.8 ± 0.02 °C) [48] at 18°C ± 0.1 nearly overlaps to those corresponding to DMPC (black squares) in the same range of hydration. However the PO_2^- frequency shows a decrease for 14:0 Diether PC at the same water content (blue circles).

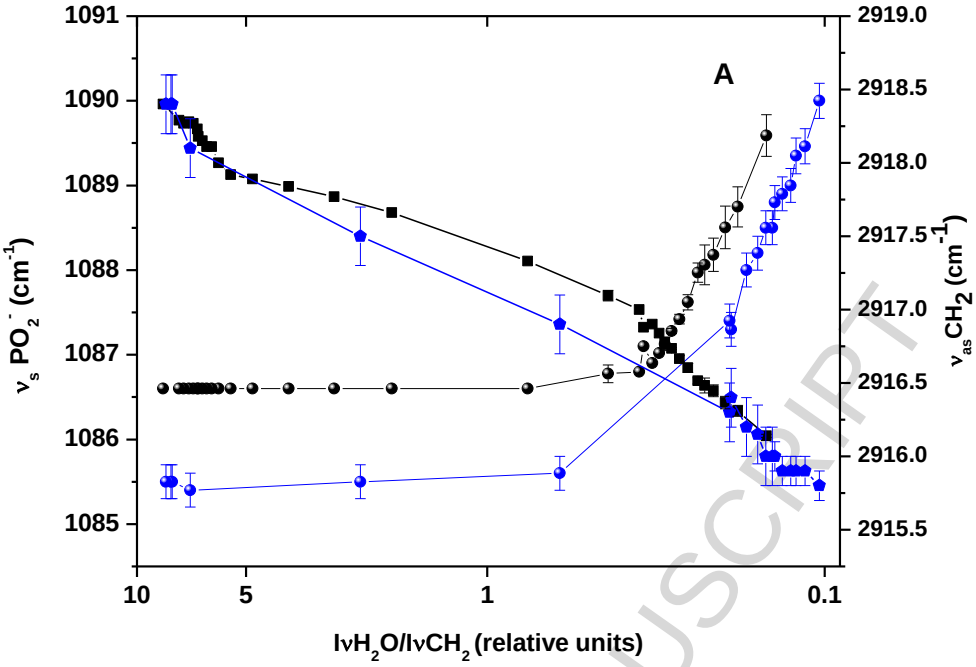

Figure 5 (A) Effect of water content on the $\nu_s PO_2^-$ (Black circles for DMPC and blue circles for 14:0 Diether PC) and $\nu_{as} CH_2$ (black squares for DMPC and blue pentagon for 14:0 Diether PC) at 18 °C ± 0.1.

The effect of the phase transition on the $\nu_{as} CH_2/\nu_s PO_2^-$ profile for Ether and Ester PC is now described in Figure 5B. At low water content, $\nu_{as} CH_2$ is lower for DMPC at 18 °C with respect to 35 °C. This slight difference could be, in principle, ascribed to a thermal effect on the CH_2 vibrational modes. When water content increases, the $\nu_{as} CH_2$ at 18 °C are always below those at 35 °C. In addition, the phosphate values at 18 °C saturate at a higher frequency meaning a lower water content than that at 35 °C.

The same analysis for 14:0 Diether PC gives the same picture: the frequencies of CH_2 at 18 °C are always below that at 35 °C in the whole range of hydration. However, at the high hydration region, phosphate frequencies, both at 18 °C and 35 °C, are displaced to lower values in comparison to DMPC for the same temperatures.

The absence of carbonyl groups at 18 °C produces a decrease in the CH_2 frequency (full blue circles) in the whole range of PO_2^- frequencies in comparison to DMPC (orange full squares) denoting a higher packing of the acyl chain congruent with the

slight increase in the transition temperature [48] of 14:0 Diether PC. The same effect is observed at 35 °C (blue empty circles and orange empty squares).

In addition, the lower values of $\nu_s PO_2^-$ for 14:0 Diether PC in comparison to DMPC both at 18 ºC and 35 °C, indicate that these groups are more exposed to water in the absence of carbonyls or that a different organization of water is displayed.

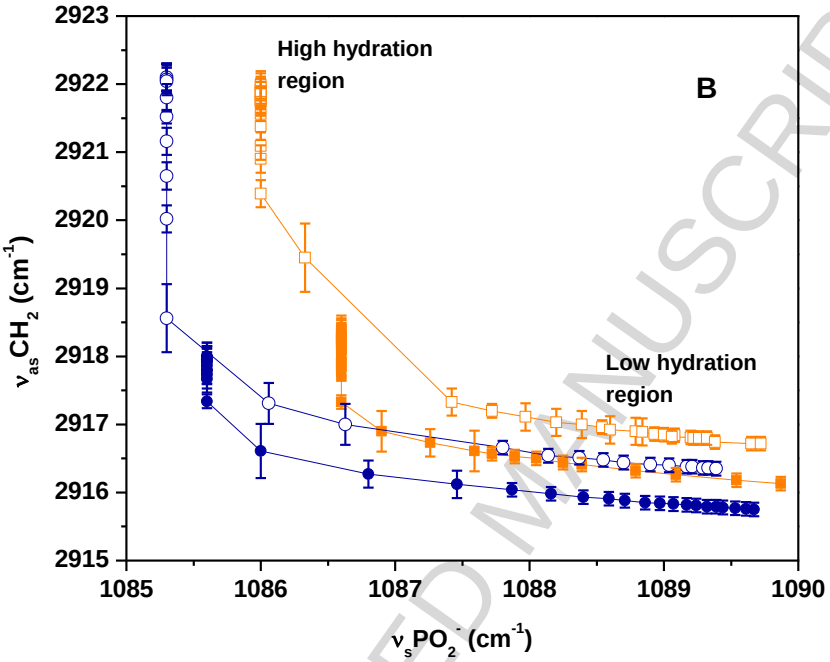

Figure 5: (B) Correlation of $\nu_s PO_2^-$ and $\nu_{as} CH_2$ equilibrated at 11% RH for DMPC at 35 °C (orange empty squares), DMPC at 18 °C (orange full squares), 14:0 Diether PC at 35 °C (blue empty circles) and 14:0 Diether PC at 18 °C (blue full circles).

Finally, in order to inspect the effect of the head group on the correlation of PO_2^- and CH_2 hydration, the profiles for 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE) were analysed at 11% RH

Data in Figure 6 denote that dehydration of DMPE consists of several stages that were not observed in DMPC. In stage I, the acyl chains are strongly affected (blue empty circles) while the $\nu_s PO_2^-$ remains constant (See arrow) around 1075.0 cm^{-1} (blue full squares); In stage II, negligible variations between $\nu_{as} CH_2$ and the $\nu_s PO_2^-$ were observed. In this step, further dehydration is required to observe significant changes in both groups. In Stage III the behaviour of $\nu_{as} CH_2$ follows a similar pattern than in DMPC at 18 °C (Figure 5A). However $\nu_s PO_2^-$ frequencies decrease

significantly in comparison to DMPC at 18 °C. This decrease indicates a weakening of the PO bond that could be related to an increase of hydrogen bonds between phosphates and the surroundings.

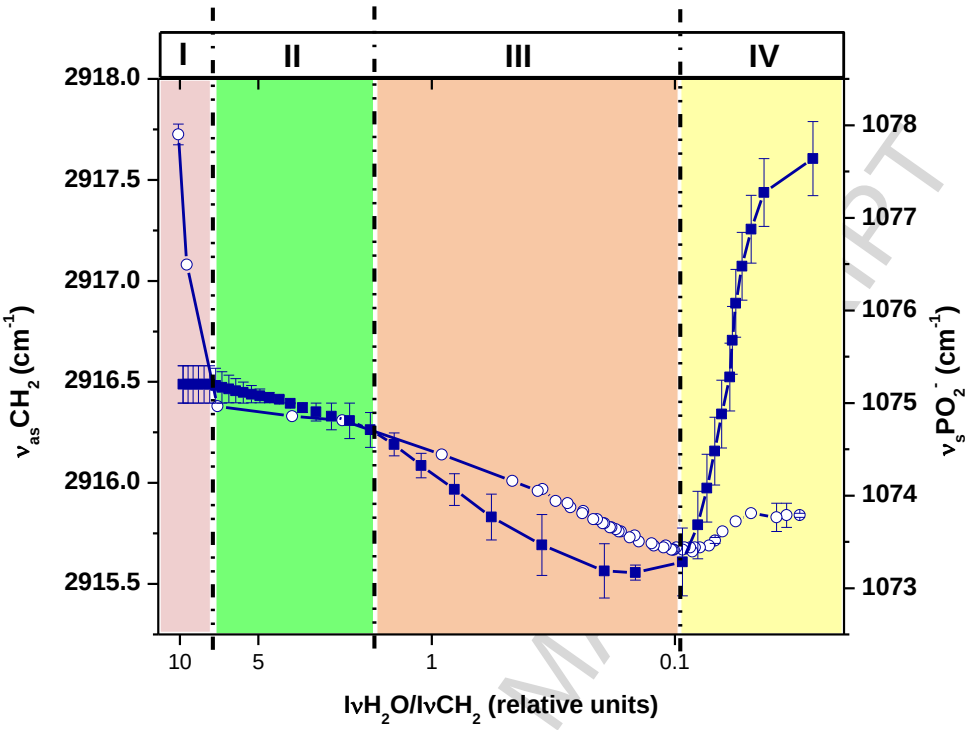

Figure 6: Effect of water content on the $\nu_s PO_2^-$ (blue squares) and $\nu_{as} CH_2$ (blue circle) of DMPE at 22°C equilibrated at 11% RH

This could be, in principle, due to the dehydration of the amine group enhancing the interaction of amine group with phosphate. The last stage (IV) is qualitatively similar to that found for DMPC (Figure 5A). However lower phosphate frequency values were found for PE in spite of the much lower level of water content than in PC. This reflects the strong lateral interactions of adjacent lipids between NH_3 and PO_2^- groups present in the PEs [23].

DISCUSSION AND CONCLUSIONS

Currently, two kinds of water in a lipid membrane are considered: a tightly bound water shell (hydration water of the head groups) and loose water beyond that hydration shell [32, 49]. This last one depends on the phase state and the lateral pressure of the membranes. In correlation with this observation, it has been reported

that water bands differ qualitatively in lipids in the gel and in the liquid crystalline state [45], indicating that also changes in water organization are feasible at the lipid phase transition (See Figure S3). These types of water may be distributed along the lipid molecule and form regions of different organization and hence with different energetic states [50].

The interaction of different types of molecules with the membrane structure may take place in different stages. One of them could be a dehydration step of the molecule and/or the membrane previous to the attachment to a particular group. Thus, mechanisms of interaction may involve displacements or rearrangements of water from or at different sites according to the energetic of the process.

The adsorption isotherms obtained for different compounds and types of membrane show different degrees of complexities from single independent sites (Langmuir isotherms) to complex ones assumed to be caused by an interdependence between the adsorption sites. This last case is phenomenological interpreted as processes of adsorption involving cooperativity and synergisms.

The present results provide a good insight for the analysis of the cooperativity that may occur between the hydration sites during adsorption processes, in the sense that the occupation of PO_2^- and/or acyl region is different according to the phase state, chain length, presence of carbonyls, type of head group and double bonds.

The importance of determining different water populations in different sites in the lipid interphase and their correlations is mainly linked to the acceptance that the role of water in lipid membrane is not only a structural component but it also has differential thermodynamic properties [30, 33]. These features would determine membrane response since those structures affect the free energy states of the different topological surface arrangements. In this regard, hydration centres may show different arrangements of the H-bond pattern of water with itself and with the membrane groups. Accordingly, labile water states can be exchanged in adsorption, partition or penetration processes of compounds of biological interest as peptides, free aminoacids, aminoacid residues of proteins, and so on [39, 40, 51]

In a simplified scheme, bulk water with a tetrahedral array must reorient in the presence of a surface. Since hydration sites are of different nature, they organize water in different energy states and structures: phosphates groups in which water molecules should orient with its positive ends (protons); carbonyl groups with electron pairs oriented in 120^o and hydrophobic groups (cholines and hydrocarbon

chains) in which water is compelled to reinforce the tetrahedral array of the bulk water [25]

The stabilization of these arrangements requires a matching that may force a distortion of the hydrogen bonds of water with those lipid residues, and thus an excess of surface energy would accumulate. Therefore, the energy of water in each site will be different and in correlation with the interaction of the adjacent residues. As shown, the dehydration process, involved in a solute-membrane interaction, can present several combinations: dehydration of phosphates according to the presence or not of carbonyl groups; the dehydration/hydration of acyl chain depending on the presence of choline or ethanolamine groups and the presence of double bonds. This several interplay can be done between CO, PO_2^- and acyl chains, giving as a result, a highly dynamic heterogeneous surface. The present analysis shows that the phosphate hydration is coupled to CH_2 hydration and that it is modulated by double bonds, CO groups and in some extent by the acyl chain length for each membrane phase state.

Are then, dynamics and thermodynamics of lipid response governed by water arrangements?. In general, the features of interfacial water are considered in relation to binding and reactivity properties of surfaces. In this sense, water in reactive sites should have a more rapid *dynamic* than hard core hydration water and bulk water, in terms of reorientation and diffusional properties.

The dynamics of interfacial water relaxation is strongly linked to the presence of surfaces [52]. Surface pressure and water ratio are coupled phenomena in expansion-contraction processes and in osmotic (permeability) processes. The correlation of the surface pressure with the surface packing would affect the dynamics of water exchange [53]. Thus, the different kinds of structured water found according to the surface features offer different levels of free energy accumulation with implicantions on membrane response.

Given the chemical and topological variability of a lipid interphase due to composition and lateral pressure, water levels may vary locally and in multiple arrays. Local curvature and protrusions would play a major role in the water dynamics and hence in biological processes and, most importantly, when environmental levels of water are changed. This paper opens the discussion for further analysis in regards to the role of water in membranes along physiological processes and states.

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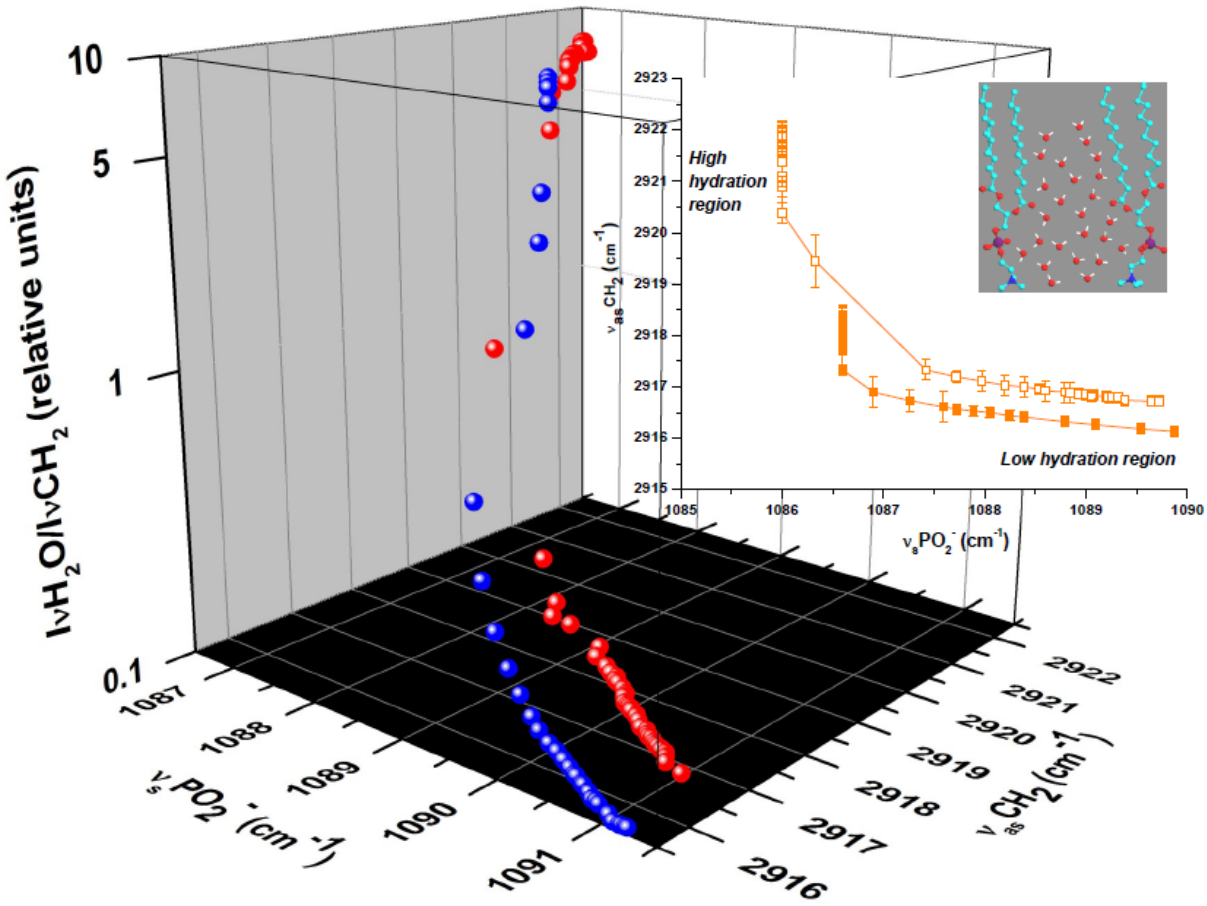

Graphical abstract

Highlights

.-Water intercalates in the acyl chain region of lipid membranes correlated with the hydration of the phosphate groups.

.-The correlation is sensible to the chain length, the presence of double bonds and the phase state of the lipid membrane.

.-Carbonyl groups CO modifies the profile of hydration of the two regions.

.-Different water populations in each condition.